REMARKS

This Amendment is in response to the Office Action, dated October 15, 2009 ("Office Action"). Claims 1-10 and 12-23 are pending; claims 1, 7, 10, 16, and 21 having been amended, and claim 11 having been canceled by virtue of the present amendment. No new matter has been added. Allowance and reconsideration of the application in view of Applicants' amendment and the ensuing remarks are respectfully requested.

The specification was amended to correct an obvious typographical error in the formula. "Pn" was corrected to "P^n" and "(1-P)(X-n)" was corrected to "$(1-P)^{(X-n)}$" as the superscript was inadvertently removed. No new matter is added. As recognized by the Examiner, Whitelegge et al., Phytochemistry 56 (2004) 1507-1515 is the journal publication of the present invention. Whitelegge et al. correctly represent the Isosolv formula, which is: $prob(n) = combin(X,n) * P^n * (1-P)^{(X-n)}$.

Claim 1 has been amended to indicate that the method includes "performing expression proteomic analysis on the organism or sample with the analysis of said subtle isotope modification." No new matter is added. Support for this amendment may be found throughout the specification.

Claims 7, 16, and 21 have been amended to correct obvious errors and thus, the amendment changes "$^{13}C:^{12}C$" to "$^{12}C:^{13}C$" and "200:1" to "100:2." No new matter is added. Support for this amendment may be found throughout the specification; for example, page 6, lines 31-34; and page 14, lines 11-13.

Claim 10 has been amended to indicate that an organism in which a subtle isotope modification has been induced is "to perform expression proteomic analysis on the organism"; and that an analytic tool to analyze said subtle isotope modification is "configured to analyze turnover of peptides, polypeptides or both." No new matter is added. Support for this amendment may be found throughout the specification.

In the Office Action, claims 19-23 were rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement for

reasons of record. Particularly, the Examiner alleged that the entirety and the parameters of Isosolv algorithm are not fully disclosed. Applicants respectfully traverse this rejection.

The determination as to whether one skilled in the art would recognize that the applicant was in possession of the claimed invention as a whole should include, among other things, the level of skill and knowledge in the art. *See* "Written Description Training Materials, Revision 1, March 25, 2008," page 1.

The Isosolv algorithm uses standard techniques to estimate the isotope envelope for a compound given the proportions of the different isotopes for each element in that compound. These standard techniques are known in the art; for example, Mass Spectrometry by J H Gross, pp 74-87, or various tools available on the Internet such as, Isotope and IsoPat.

The Isosolv algorithm equation is as follows:

$$prob(n) = combin(X,n) * P^n * (1-P)^{(X-n)}.$$

Applicants respectfully submit that one of skill in the art will readily appreciate that the parameters of the equation are as follows:

prob(n) is the probability that the molecule will be made up of n atoms that are not the most abundant (e.g., ^{13}C) (see example 7, "the probability of having 'n' ^{13}C's");

"combin" is a common spreadsheet/calculator name for the function to calculate combinations. The equation to calculate "n choose K" (i.e., combin(n,k)) is: $n! / k!(n-k)!$).

X is the total number of atoms of that element in the molecule (see example 7, "'X' total carbon atoms";

P is the probability of the rare isotope (see example 7, "a ^{13}C probability of 'P'"); and

n is the number of atoms that are not of the most abundant isotope (see example 7, "'n' ^{13}C's").

Including the impact of the other naturally occurring isotopes (e.g., ^{18}O) is merely a matter of combining the probabilities.

Isosolv is not claiming a method to calculate isotope distributions. Isosolv is an algorithm to estimate, from measured isotope abundances, the amount of a particular isotope in an analyte. One of skill in the art will readily know that this is performed by

iteratively calculating theoretical mass spectra that would result of differing probabilities for a given isotope until a best fit mass spectrum is obtained. The probability for the isotope that yields the best fit is then returned.

Accordingly, Applicants respectfully submit that the specification provides sufficient written description to one of skill in the art to analyze a subtle isotope modification with the Isosolv algorithm. In light of the foregoing, Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1-6 and 8-23 were rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for reasons of record. Particularly, the Examiner asserted one of skill in the art would not know the metes and bounds of the term "subtle" as recited by claim 1. Applicants respectfully traverse this rejection.

Applicants submit that one of skill in the art will readily appreciate the metes and bounds of the term "subtle" as defined in the specification and used in the claims. As the Examiner concedes, the specification provides a definition for "subtle," including that "there is a measureable effect upon the observed peptide isotope distribution, without causing a gross extension or displacement of the single isotope envelope." (See Specification page 5, lines 28-32) As the definition provides, the minimal amount of change is that amount that produces a "measurable effect upon the observed peptide isotope distribution." One of skill in the art is able to ascertain when an isotope modification results in a measurable effect; for example, by analyzing a sample with a mass spectrometer. Therefore, the minimal amount of change is an amount that produces an effect on the observed peptide isotope distribution that can be measured. One of skill in the art will also know that a change that induces "a gross extension or displacement of the single isotope envelope" will not be considered subtle. For instance, in Figure 1, there is a single isotope envelope in each mass spectrum, whereas the prior art of record shows a displacement of the single isotope envelope. Figure 3 of Oda *et al.* shows two isotope envelopes; one for ^{14}N and one for ^{15}N. Figure 3 of Ong *et al.* also shows two isotope envelopes; one for Deu-d0 and one for Deu-d3.

Paša-Tolić *et al.* also shows two isotope envelopes in Figure 1B; a peak at the left is the displaced envelope as this peak is created by the depleted sample. As such, once there is a gross extension or displacement of the single isotope envelope, the modification will no longer be considered subtle. In light of the forgoing remarks, Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 19-23 were rejected under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the enablement requirement for reasons of record. Applicants respectfully traverse this rejection.

It is well-established that the factors in determining whether undue experimentation is required are summarized in *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988). The court in *Wands* states, "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." *Id.* at 1404. The factors to be considered include: (1) the breadth of the claims; (2) the nature of the invention; (3) the state of the prior art; (4) the relative skill of those in the art; (5) the predictability or unpredictability of the art; (6) the amount of direction or guidance presented; (7) the presence or absence of working examples; and (8) the quantity of experimentation necessary to practice the invention.

Applicants submit that (1) the breadth of the claims is clearly defined as explained above. In sum, one skilled in the art will readily appreciate the metes and bounds of a "subtle" isotope modification. Further, the Isosolv algorithm and its parameters are provided in the application and one of skill in the art can readily understand how to use the Isosolv algorithm. Thus, the breadth of the claims is fully supported by the instant application.

Applicants submit the (2) nature of the invention is an algorithm to estimate, from measured isotope abundances, the amount of a particular isotope in an analyte. One skilled in the art will readily know that this is performed by iteratively calculating theoretical mass spectra that would result of differing probabilities for a given isotope until a best fit mass spectrum is obtained. The probability for the isotope that yields the

best fit is then returned. Isosolv is not claiming a method to calculate isotope distributions.

Applicants submit that (3) the state of the prior art is such that one of skill in the art is able to use standard algorithms and standard mathematical techniques to perform calculations according to an algorithm. Isosolv is an algorithm to estimate, from measured isotope abundances, the amount of a particular isotope in an analyte. One of skill in the art will readily be able to iteratively calculate theoretical mass spetra that would result in different probabilties for a given isotope until a best fit mass spectrum is obtained. The Isosolv algorithm uses <u>standard</u> techniques to estimate the isotope envelope for a compound given the proportions of the different isotopes for each element in that compound. These <u>standard</u> techniques are known in the art; for example, Mass Spectrometry by J H Gross, pp 74-87, or various tools available on the Internet such as, Isotope and IsoPat.

Applicants submit that (4) the relative skill of those in the art is high; and (6) the amount of direction or guidance presented in the application is sufficient to one of skill in the art. Additionally, (7) the presence of working examples provides adequate examples for one of skill in the art to practice the present claims. Example 7 explains the Isosolv algorithm: $prob(n)= combin(X,n) * P^n * (1-P)^{(X-n)}$ and its parameters. As discussed above, prob(n) is the probability that the molecule will be made up of n atoms that are not the most abundant (e.g., ^{13}C); "combin" is a common spreadsheet/calculator name for the function to calculate combinations (the equation to calculate "n choose K" (i.e., combin(n,k)) is: $n! / k!(n-k)!$)); X is the total number of atoms of that element in the molecule; P is the probability of the rare isotope; and n is the number of atoms that are not of the most abundant isotope. The impact of the other naturally occurring isotopes (e.g., ^{18}O) is merely a matter of combining the probabilities.

Finally, Applicants submit that (8) the quantity of experimentation required to practice the present claims is not undue. "A considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 858 F.2d 731, 737, 8 U.S.P.Q.2d 1400, 1404. Applicants respectfully submit that one of skill in the art could fully practice the

present claims without undue experimentation and with a reasonable expectation of success. The details of Isosolv are given in the specification. Again, the Isosolv algorithm is to estimate, from measured isotope abundances, the amount of a particular isotope in an analyte and this is performed by iteratively calculating theoretical mass spectra that would result. Iterative calculations for estimations are routine in the art and thus, not undue.

In light of the foregoing, Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1-8 and 10-17 were rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Ford et al. (Biomedical Mass Spectrometry, 1985) for reasons of record. Particularly, the Examiner asserts that Ford et al. discloses isotope enrichment of ^{13}C leucine in human. The Examiner states that the claims' recitation of "performing expression proteomic analysis" was not given patentable weight because the recitation occurs in the preamble. Applicants respectfully traverse this rejection.

Applicants submit that Ford et al. discloses a tracing an amino acid and as the Examiner acknowledges, does not perform expression proteomic analysis. While Applicants in no way concede to the merits of the Examiner's rejection, the claim 1 has been amended to recite that the method involves "performing expression proteomic analysis on the organism or sample with the analysis of said subtle isotope modification." Claim 10 has been amended to indicate that an organism in which a subtle isotope modification has been induced is "to perform expression proteomic analysis on the organism"; and that an analytic tool to analyze said subtle isotope modification is "configured to analyze turnover of peptides, polypeptides or both." Ford et al. does not teach these elements. Accordingly, Ford et al. does not anticipate these claims. In light of the foregoing, Applicants respectfully request reconsideration and withdrawal of this rejection.

Claims 1, 3, 4, 6, 8-10, 12, 15, 17, and 18 were rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Ong et al. (Molecular and Cellular Proteomics, 2002) for reasons of record. Particularly, the Examiner asserts that Ong et al. teaches the use

of deuterated leucine in methods and systems to perform stable isotope expression analysis of proteins. The Examiner also asserts that Ong *et al.* discloses that 99% deuterium labeled and unlabeled leucine are used to isotopically label protein samples in mammalian cells and mixed together at concentrations of 1:1 and 1:3; that the proteins were analyzed by MALDI-TOF mass spectrometry; and that the ratios of the isotopic distributions of peptides were determined. Again, the Examiner states that the claims' limitation of "performing expression proteomic analysis" was not given patentable weight because the recitation occurs in the preamble. Applicants respectfully traverse this rejection.

Applicants respectfully submit that Ong *et al.* discloses the full isotope exchange, that is, to swap all leucines for deuterated leucines (Leu-d3). (See Abstract.) According to the experimental procedures, the cell lines were grown in labeling media containing *either* normal leucine (Leu-d0) *or* Leu-d3. (See page 377, second full paragraph; see also Figure 1.) It is during the relative quantitation of protein expression that mixtures of Leu-d0 and Leu-d3 labeled samples are made. (See page 377, fifth full paragraph.) That is, the cells were not grown in media with mixtures of Leu-d0 and Leu-d3. As such, Ong *et al.* discloses the full isotope exchange and not subtle isotope modification. Accordingly, Ong *et al.* does not meet a feature of the claim that requires the inducement of a subtle isotope modification.

Additionally, claim 1 has been amended to recite that the method involves "performing expression proteomic analysis on the organism or sample with the analysis of said subtle isotope modification." Claim 10 has been amended to indicate that an organism in which a subtle isotope modification has been induced is "to perform expression proteomic analysis on the organism"; and that an analytic tool to analyze said subtle isotope modification is "configured to analyze turnover of peptides, polypeptides or both." Ong *et al.* does not teach these elements. However, Applicants in no way concede to the merits of the Examiner's rejection.

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the §102(b) rejection.

Claims 1-3, 6, 8-12, 15, and 17-18 were rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Paša-Tolić et al. (JACS, 1999) for reasons of record. Particularly, the Examiner asserts that Paša-Tolić et al. teaches modification of the stable isotopes of an organism by depleting ^{13}C, ^{15}N and ^2H by growth in a stable isotope medium. The Examiner reasons that the natural abundance of these isotopes is very low, so the change in their abundance is subtle. Applicants respectfully traverse this rejection.

Applicants respectfully submit that the Examiner has incorrectly interpreted the modification made by Paša-Tolić et al. The natural abundance of ^{13}C is about 1.1%. In Applicants invention, changing the ratio of ^{13}C to ^{12}C by inducing 1.5%, 3.0% or 6.0% change is increasing the abundance of ^{13}C to a total of 1.5%, 3.0% or 6.0% (i.e., 1.5/100, 3.0/100, or 6.0/100). The change with respect to naturally occurring ^{13}C is quite small; for example, going from about 1.1 to 1.5, 1.1 to 3.0 or 1.1 to 6.0. The increase of ^{13}C is subtle because the change in abundance ^{13}C in the sample is very small. At first blush, Paša-Tolić et al. might appear to be inducing a subtle modification since it decreased ^{13}C from its natural abundance of about 1.1% to about 0%. However, this decrease must be viewed from the perspective of the change in the abundance of ^{13}C in the sample. The change in the abundance of ^{13}C by Paša-Tolić et al.'s modification is quite large because the elimination of ^{13}C is not a 1.1% decrease of ^{13}C in the sample, it is a 100% decrease of ^{13}C in the sample. Thus, Paša-Tolić et al. does not teach a subtle modification.

Further evidence is provided by Figure 1B in Pasa-Tolic et al., which shows two isotope envelopes; a peak at the left is the displaced envelope as this peak is created by the depleted sample. As such, once there is a gross extension or displacement of the single isotope envelope, the modification is not subtle. Accordingly, Paša-Tolić et al. does not anticipate these claims. In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the §102(b) rejection.

Claims 1-3, 6, 8-12, 15, and 17-18 were rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Oda et al. (PNAS, 1999) for reasons of record. Particularly, the Examiner asserts that Oda et al. teaches labeling yeast cells with ^{15}N

by growth in a stable isotope medium with 99.6% labeling. Applicants respectfully traverse this rejection.

Applicants submit that Oda *et al.* does not teach inducing a subtle isotope modification. In fact, Oda *et al.* teaches the use of a use of greater than 96% 15N media to grow the cells, which is not subtle. (See page 6592 under "Quantitation of Changes in Protein Expression.") Similar to Ong *et al.*, the pools of cells are combined, the cells were not grown in a combination of "normal" media and "enriched" media. In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the §102(b) rejection.

Claims 1-18 were rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over Ford *et al.* in view of Oda *et al.* The Examiner, however, discussed Paša-Tolić *et al.* in the body of the rejection and not Oda *et al.* Particularly, the Examiner asserts that Ford *et al.* discloses the determination of isotope enrichment of ^{13}C leucine in human plasma by mass spectrometry. The Examiner admits that Ford *et al.* performs analysis only on a single amino acid and does not analyze peptide or protein turnover, and that Ford *et al.* does not use MSMS for the analysis. The Examiner asserts that Paša-Tolić *et al.* discloses performing proteomic expression analysis and recognizes that the technique is applicable for higher order organisms such as mammals and humans. The Examiner asserts that Paša-Tolić *et al.* uses MSMS technique. Thus, the Examiner concluded that it would have been prima facie obvious to one of skill in the art to use MSMS technique because Paša-Tolić *et al.* indicates that the MSMS technique would be useful in higher organisms. Applicants respectfully traverse this rejection.

"[t]he mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art." MPEP §2143.01 (citing KSR International Co. v. Telefex Inc., 550 U.S. 398, 82 USPQ2d 1385 (2007)). As such, a "reasonable expectation of success is required." MPEP §2143.02.
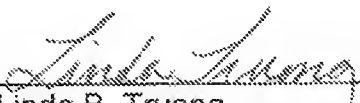
Applicants request clarification on which prior art references are being applied and how they are being applied; particularly, with respect to Paša-Tolić *et al.* and Oda *et al.*

In an effort to advance prosecution, Applicants assume that all three are applied and offer the following remarks. As discussed above, Ford *et al.* discloses tracing an amino acid, and as the Examiner acknowledges, does not perform expression proteomic analysis. While Applicants in now way concede to the merits of the Examiner's rejection, claim 1 has been amended to recite that the method involves "performing expression proteomic analysis on the organism or sample with the analysis of said subtle isotope modification." Claim 10 has been amended to indicate that an organism in which a subtle isotope modification has been induced is "to perform expression proteomic analysis on the organism"; and that an analytic tool to analyze said subtle isotope modification is "configured to analyze turnover of peptides, polypeptides or both. Also discussed above, Paša-Tolić *et al.* does not disclose subtle isotope modification as it teaches a 100% decrease of the rare isotope. Additionally, Paša-Tolić *et al.* did not use MSMS in its analysis. Also as discussed above, Oda *et al.* does not teach inducing a subtle isotope modification because it teaches use of a use of greater than 96% ^{15}N media to grow the cells, which is not sublte. The pools of cells are combined; the cells were not grown in a combination of "normal" media and "enriched" media.

Consequently, one of ordinary skill in the art would not combine Ford *et al.*, Paša-Tolić *et al.* and Oda *et al.* Even if the combination is proper, which Applicants do not concede to, the combination would not render the claims obvious because the result would not have been predictable. Ford *et al.* merely teaches tracing an amino acid, it provides no teaching or predictability regarding expression proteomic analysis. Paša-Tolić *et al.* and Oda *et al.* do not teach subtle modification. Without the teaching of subtle modification for use in proteomic analysis, one of skill in the art would not enjoy a reasonable expectation of success because the result of the subtle modification would not have been predictable. In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the §103(a) rejection.

All of the claims remaining in the application are now believed to be allowable. Favorable consideration and a Notice of Allowance are earnestly solicited. If questions remain regarding this application, the Examiner is invited to contact the undersigned at (213) 633-6800.

Respectfully submitted,
Julian P. Whitelegge *et al.*
DAVIS WRIGHT TREMAINE LLP

By _____
Linda B. Truong
Registration No. 56,461

865 South Figueroa Street, Suite 2400
Los Angeles, CA 90017-2566
Phone: (213) 633-6800
Facsimile: (213) 633-6899